

The University of Chicago

EFFECT OF SOME ENVIRONMENTAL
FACTORS ON FLORAL INITIATION
IN XANTHIUM

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF BOTANY

1940

By
LOUIS K. MANN

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EFFECT OF SOME ENVIRONMENTAL FACTORS
ON FLORAL INITIATION IN XANTHIUM¹
CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 517

LOUIS K. MANN
(WITH FIVE FIGURES)

Introduction

Attention has recently been called to the fact that there are effects attributable to both the dark period and the photoperiod in the initiation of flower primordia and the development of flowers resulting from photoperiodic treatment. This interpretation has been applied to the reactions of short-day plants. In the cocklebur (*Xanthium pennsylvanicum*) and the Biloxi soybean (*Soja max*), the conditions prevailing during either the dark period or the photoperiod may be a limiting factor in the induction of flowers (2). For example, *Xanthium* will flower after exposure to a 12-hour dark period following a photoperiod of high-intensity light. Similar plants will remain vegetative if the photoperiod preceding this dark period is of very low light intensity. But, regardless of the length of the photoperiod or the intensity of the light, a continuous dark period of at least $8\frac{1}{2}$ –9 hours is necessary for the induction of flower primordia.

In an endeavor to provide a brief formulation of the fact that both the photoperiod and the dark period are concerned in the ultimate conditions necessary for floral initiation and development, HAMNER (2) has suggested that their relationship may be represented as follows: $A, B \rightarrow C$, in which A represents the changes or conditions which arise owing to exposure to light; B, those owing to darkness; and C, the possible summation or resultant changes related to A and B. These same terms are used here, but it is not assumed that definite, known reactions are represented by them. The physical or chemical changes or conditions constituting A, B, and C are still to be determined. Although there is some evidence that diffusible substances are involved (3), up to now attempts to isolate flower-inducing substances have not demonstrated either their actual occurrence or their nature.

Following specific photoperiodic treatments, conditions A, B, and their possible resultant, C, may become evident through the initiation and development of flower structures. In so far as A, B, and C pertain to floral initiation and develop-

¹ This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. Part of the equipment was obtained through a grant to Dr. K. C. Hamner from the American Association for the Advancement of Science.

ment, the degree of initiation and development may be used as an indicator of the intensity of these conditions, although it is generally recognized that there are definite responses by other parts of the plant as well. In the following experiments conditions of light intensity and temperature were carefully controlled, and an estimate of the intensity of A and B as conditioned by these factors is assumed to be indicated by the degree of floral initiation and development.

Material and methods

Experiments 1-4 and 8 were carried out in March, April, and May, 1940, at the U.S. Horticultural Station, Beltsville, Maryland. Seeds of *Xanthium pennsylvanicum* were collected at Chicago in the autumn of 1939 and stored out-of-doors during the winter. The seeds were planted in flats, the seedlings later transplanted to 3½-inch pots, and most experimental treatments begun when the plants—carefully selected for height and vigor—were 40 days old. They were kept on greenhouse benches receiving natural daylight, which was extended from sundown until 2:00 or 3:00 A.M. by about 100 foot-candles of Mazda filament light.

The specific treatments usually required 4-5 days for completion, after which time the plants were returned to the greenhouse for the duration of the experiment, at the end of which the stage and type of development of the terminal bud of all plants were determined by means of a low-power binocular microscope. All dissections, unless otherwise noted, were made 22 days after the conclusion of treatment. Dissections of control plants were made before and after experiments to insure that conditions of long photoperiod had been maintained; that is, that plants which received no special treatment remained strictly vegetative. The plants grew vigorously, most of them being over 3 feet high at the time the terminal bud was dissected. Because of the limited amount of soil in each pot, the plants were watered with complete nutrient solution on alternate days from the time the treatment was completed until dissection.

The character of experiments 1-4 made necessary an extensive scale for recording floral stages, the frequently used divisions into inflorescence primordia, flower primordia, etc., not being sufficiently precise to indicate the several stages recorded. The degree of initiation or development of flowers in a plant was designated by a number. The stage of floral development of strictly vegetative plants was designated as zero. In plants not strictly vegetative the number was, in general, equivalent to the transverse diameter of the terminal staminate inflorescence measured in units of 0.1 mm. For diameters below 0.7 mm. the diameter alone was not an adequate measure, and six stages—based mainly on appearance—were recognized. An inflorescence with a diameter of less than 0.7 mm. usually possesses few, if any, flower primordia, but consists for the most part of a smooth,

bulbous inflorescence axis surrounded at the base by several primordia of bracteal leaves. When an inflorescence has reached the diameter of 0.7 mm. there are usually one or more cycles of flower primordia around the basal portion of the axis. As the inflorescence continues to develop, primordia appear in acropetal succession until the axis is completely covered, a condition found in almost all inflorescences of a diameter of 1.2 mm. or larger. For any given inflorescence diameter within the range 0.7–1.2 mm., the average relative portion of the axis covered with flower primordia was determined. If in any plant with an inflorescence diameter within this range the relative portion of the enlarged axis tip covered with primordia was greatly different from the average for that diameter, the measured diameter was modified slightly. For inflorescences with a diameter greater than 1.2 mm. the diameter was used directly. Since the environment during any experiment affects initiation and rate of floral development, the stage is of comparative value and its use is justifiable only within a single experiment, or in experiments conducted under identical conditions.

For any specific treatment within an experiment, a unit which usually consisted of ten or more plants was used. When all plants of a unit were flowering (no vegetative plants present), the average of their floral stages presents a fair estimate of the resultant effects of the stimulus. If, however, a unit was given a photoperiodic treatment of just sufficient strength to bring about the first recognizable stage of flowering, the variability in the response of these plants is such that approximately half of them would show various stages of flowering while the other half would remain vegetative. Among the vegetative plants, some would require only slight additional stimulus to bring about initiation of flowers, while others would require a much greater stimulus. Since these vegetative plants are all given the value zero as their stage of flowering, no negative values being used, the numerical average of the plants in the unit is much too high. As another example, suppose that following a given photoperiodic treatment one plant flowers out of a group of ten, the remainder being vegetative; the average stage of flowering of this group would be positive and equal to one-tenth the flower stage of the single flowering plant. Rather than make an arbitrary correction for these vegetative plants in the following graphs, a list of all units containing two or more vegetative plants is given in each case.

The temperature of the greenhouse was not allowed to go below 70° F., and only on a few days in May did it rise above 85° F. All experimental rooms except the darkroom were refrigeration rooms, where light intensity, temperature, and humidity were controlled. The darkroom was not refrigerated but for most experiments was maintained at 23° C. or slightly above. Each refrigeration room contained either a 60 or a 75 ampere arc light burning Everready Sunshine car-

bons. This was supplemented by Mazda filament light of between 100 and 200 foot-candles' intensity. Light intensity was controlled by shading the arc lights with thin cloth curtains and by changing the position of the benches on which the plants were placed. Temperatures were kept within 1° C. of that specified. Relative humidity was kept within 80-100 per cent.

In several of the experiments determinations of the effect of temperature, light intensity, and other factors during a single photoperiod were made. In such cases some method must be used to remove, or at least to reduce, the effect of the light which the plants previously received in the greenhouse. It has been shown (2) for *Xanthium* that exposure to repeated short cycles, each consisting of 2-3 hours of darkness followed by 2-3 minutes of medium light intensity, has this effect. Several hours of this treatment following a long bright photoperiod lowers the effectiveness of the photoperiod to the extent that the plants may be exposed to a long dark period and no initiation of flowers will result. In the experiments presented here, except as otherwise specified, a single cycle of this treatment consisted of a 1-hour dark period followed by a 3 ± 1 -minute photoperiod. The light intensity during the photoperiod was 40 foot-candles; the temperature during the entire cycle was 23° C. Exposure to several such consecutive cycles is here designated as short-cycle treatment. The cycles were usually repeated consecutively for a period of 40 hours. In some cases the control units used to test the effectiveness of the short-cycle treatments did not consist entirely of vegetative plants. However, the residual effects from previous photoperiods are lowered sufficiently so that they are probably of little significance.

As has previously been demonstrated, and as is shown in experiment 6, the duration and intensity of the light of the photoperiod immediately following a long dark period have pronounced effect on subsequent flower initiation and development. This factor was therefore controlled as much as possible. Immediately following all long dark periods the plants were placed under uniform light and temperature. Here they were exposed to a photoperiod of at least 8 hours. Following this, the plants were taken out during a period of daylight and placed on the long-day bench of the greenhouse. If, as frequently was the case, the 8-hour photoperiod did not end during daylight, it was extended until 9:00 A.M. or later of the following day, at which time the plants were removed to the greenhouse.

Experiments 5-7 were carried out at Chicago during the summer of 1939. Here the procedure was variable and will be given under each experiment. The recorded stages of floral development do not correspond with that used in experiments 1-4 and 8, but follow that previously used by HAMNER (2).

Procedure and results

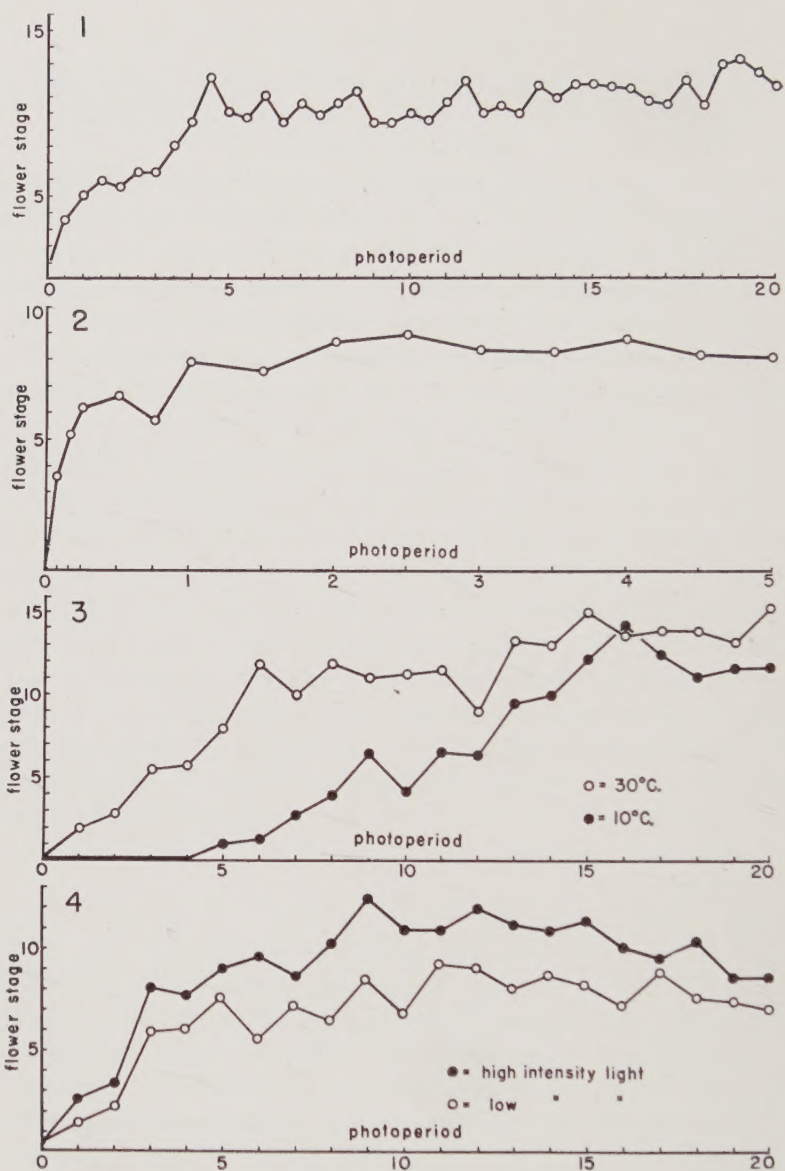
EXPERIMENT 1: INITIATION AND DEVELOPMENT OF FLOWERS AFFECTED BY LENGTH OF SINGLE PHOTOPERIOD PRECEDING LONG DARK PERIOD

Plants of *Xanthium* were given an experimental treatment consisting of a single controlled photoinductive cycle, during which induction of flowers might take place. Previous to this controlled cycle, the plants were given a short-cycle treatment. During the single photoinductive cycle all factors remained constant except the length of the photoperiod, which was varied from $\frac{1}{2}$ to 20 hours. This photoperiod, regardless of length, was followed by a 12-hour dark period.

In accordance with the general outline for experiments given before, 410 plants of a seeding of February 12 were selected from the long-day bench and placed in a control room at noon, March 12. In order to reduce the effect resulting from exposure to light in the greenhouse, the entire lot received a short-cycle treatment lasting 40 hours. Immediately following this, ten of the plants (controls) were placed in the darkroom for 12 hours. The remaining 400 plants were exposed to light of 2000–3000 foot-candles at the leaf surface, with a uniform temperature of 23° C. One-half hour later, and at each $\frac{1}{2}$ -hour interval thereafter, a unit of ten plants was transferred to the darkroom, 20 hours being required to remove all 400 plants. Thus the units received photoperiods ranging from $\frac{1}{2}$ to 20 hours. Any given unit remained in the darkroom for 12 hours, after which time it was returned to the arc room for 8 hours or more so that it could be replaced on the long-day bench during daylight. Twenty-two days after all plants had been returned to the bench, they were harvested, the terminal buds examined, and the stage of flowering recorded.

The results of this experiment are shown in figure 1. The length of the variable photoperiod following the short-cycle treatment and preceding the 12-hour dark period is indicated by the abscissa. The average of the stage of flowering of each unit is indicated by the ordinate, which may also be interpreted as indicating the intensity of condition A as affected by the length of the single photoperiod shown on the abscissa. In the lower lefthand corner is indicated the average stage of flowering of the control unit, which received no photoperiod following the short-cycle treatment or preceding the 12-hour dark period. In this unit one plant was flowering and nine were strictly vegetative. Of the other units of the experiment, none contained more than one vegetative plant.

The average of the stage of flowering increases rapidly with the increasing length of the photoperiod up to 4–5 hours. Beyond this, increasing the length of the photoperiod has much less effect on the initiation and development of flower primordia.



FIGS. 1-4.—Effect of varied lengths of photoperiod (single photoinductive cycle) on initiation and development of flower primordia in *Xanthium*: Fig. 1, of medium light intensity; fig. 2, of high light intensity; fig. 3, at two temperatures; fig. 4, at two light intensities.

EXPERIMENT 2: REPETITION OF EXPERIMENT 1, USING PHOTOPERIOD
OF HIGH LIGHT INTENSITY

This experiment was designed to determine the effect of the length of a single photoperiod preceding a long dark period on the initiation and development of flowers. The procedure differed in several respects from that of experiment 1.

Experiment 1 showed that a single $\frac{1}{2}$ -hour photoperiod of moderate light intensity preceding a long dark period is sufficient to bring about the initiation of flower primordia. In experiment 2, in which a very high light intensity photoperiod was used, several photoperiods shorter than $\frac{1}{2}$ hour were given. Another precaution was also taken. The ten control plants in experiment 1 contained one which developed primordia, indicating that there still remained a measurable effect owing to the photoperiod to which the plants had been exposed while on the long-day bench. To minimize this effect still further, the type of short-cycle treatment usually given was considerably modified.

On May 1, 267 uniform plants were selected from a seeding of March 26, which had been growing on the long-day bench, and were placed in a room where they received Mazda filament light of about 5 foot-candles for 18 hours. This treatment was followed by cycles consisting of a 2-hour dark period followed by a photoperiod of 3 ± 1 minutes of 5 foot-candles of Mazda filament light. This type of treatment was continued for 73 hours at a temperature of 19° – 20° C. Immediately following this treatment, one unit of eighteen plants (controls) was taken to a darkroom, avoiding exposure of these plants to light. This unit remained in the darkroom for 12 hours, when it was taken directly to the long-day bench. The remaining plants were divided into units of eighteen plants each. These were given varying lengths of photoperiods by exposure to direct sunlight outside the greenhouse. The light intensity remained above 10,000 foot-candles throughout the exposures; and since these were given during the early part of the day, the temperature rose from 15° C. at the beginning to 22.5° C. at the conclusion of the longest exposure. The photoperiods varied in length from 5 minutes to 5 hours (fig. 2). At the conclusion of any photoperiod, the unit was placed in a darkroom where it remained for 12 hours. After receiving the dark period, the unit was returned to the long-day bench, where all plants remained until dissected.

The data are given in figure 2. The abscissa and ordinate are similar to those in figure 1, except that the length of some of the photoperiods is different. The stage of flowering of the control unit, which received no photoperiod preceding a 12-hour dark period, is shown in the lower lefthand corner of the graph. All plants of this unit were strictly vegetative. Among the other units represented on the graph, that exposed to a 5-minute photoperiod contained five vegetative plants

out of eighteen, and the unit exposed to a 10-minute photoperiod contained three out of eighteen. No other lots contained more than one.

For a single photoperiod of high light intensity under the conditions of this experiment, the stage of flowering as here measured increased rapidly with increase in the length of the photoperiod up to about 2 hours. Increasing the length beyond 2 hours only slightly affected the stage of flowering of the plants.

EXPERIMENT 3: INITIATION AND DEVELOPMENT OF FLOWER PRIMORDIA
AFFECTED BY TEMPERATURE DURING SINGLE PHOTOPERIOD
PRECEDING LONG DARK PERIOD

Xanthium which had been grown under long-day conditions was submitted to only one photoinductive cycle, during which induction of the flowering condition might take place. As in experiment 1, preceding and following this treatment the plants were grown on the long-day bench. To reduce the effect of the photoperiods previous to the controlled photoinductive cycle, short cycles were employed. To determine the effect of temperature during the photoperiod, plants were placed in control rooms at two different temperatures, where they received photoperiods varying from 1 to 20 hours.

From plants seeded February 20 and grown under conditions of long day, 410 plants were selected. At noon, March 29, these were placed in a control room and exposed to short-cycle treatment for 40 hours. Immediately following this treatment a unit of ten plants (controls) was placed in the darkroom. The remaining 400 plants were divided into two groups of 200 each, and each group was placed in one of two control rooms, one maintained at 30° C. and one at 10° C. In both rooms the light intensity was 2000–3000 foot-candles at the leaf surface. Beginning the first hour after the plants were placed in the light rooms, units of ten plants were removed hourly from both rooms to the darkroom. Any given unit remained in the darkroom for 12 hours, after which it was returned to a control room maintained at 23° C. and having 2000–3000 foot-candles of light. After 8 hours or more in this control room the units were removed during the day to the long-day bench, where they remained until dissection.

Figure 3 gives the results of this experiment. The stage of flowering of the control unit of nine plants (one died) is shown in the lower lefthand corner of the graph. Two of these nine plants showed the first stages of flowering; the remaining seven were strictly vegetative. Of the other units in which there was more than one vegetative plant, the following list gives the lengths of the photoperiods to which they were exposed and the number of vegetative plants in each case. Among the units at 30° C., the following showed more than one vegetative plant: 1 hour, six vegetative; 2 hours, four vegetative; 3 hours, three vegetative; 4 hours, three vegetative; 5 hours, three vegetative. Among the units at 10° C., the following

contained more than one vegetative plant: 1 hour, nine vegetative; 2 hours, nine vegetative; 3 hours, all vegetative; 4 hours, all vegetative; 5 hours, three vegetative; 6 hours, three vegetative; 7 hours, three vegetative; 10 hours, five vegetative; 12 hours, two vegetative.

At a temperature of 30° C. the response of the stage of flowering to the length of the photoperiod is much like that found in experiment 1; there is a marked rate of increase in the stage of flowering with increase in the length of photoperiod up to about 6 hours. Lengthening photoperiods beyond this increases their effectiveness only slightly. At a temperature of 10° C. a photoperiod shorter than 4-5 hours had little effect so far as initiation and development of flowers were concerned.

The ordinate in figure 3 may also be interpreted as an indicator of the intensity of condition A developed by photoperiods of different lengths and at two different temperatures. In this case the level of condition A rose much more slowly in plants exposed to a photoperiod at 10° C. than in plants exposed to a photoperiod at 30° C. At 10° C., with the light intensity and quality used in this experiment, exposures to light must be 13 hours or more to bring A to the same level reached during a photoperiod of about 6 hours at 30° C. It is evident that, at least for *Xanthium*, the temperature during the photoperiod of a photoinductive cycle has a pronounced effect on subsequent initiation and development of flowers.

EXPERIMENT 4: INITIATION AND DEVELOPMENT OF FLOWERS AFFECTED BY INTENSITY OF LIGHT DURING SINGLE PHOTOPERIOD PRECEDING LONG DARK PERIOD

Following the procedure of experiment 3, the selected plants were exposed to a single photoinductive cycle during which induction of flowers might take place. Preceding and following this cycle the plants were grown under conditions of long photoperiod. The plants were divided into three lots, which were placed in separate rooms, each being exposed to a different light intensity during the single controlled photoinductive cycle. In each room the respective lots were exposed to photoperiods ranging from 1 to 20 hours. The final stage of flowering of any given unit is thus a function of the length of the photoperiod and of its light intensity.

On April 22, 610 plants of a seeding of March 13 were selected from the long-day bench of the greenhouse. To reduce the effect of the previous photoperiods, the plants were placed in a control room where they received 40 hours of short cycles. Following this treatment one unit of ten plants (control) was taken immediately to the darkroom, where it remained for 12 hours. The remaining 600 plants were divided into three lots of 200 each, which were distributed to the three arc-light rooms, all of which were kept uniformly at 23° C. In each room the plants were exposed to a different intensity of light at the leaf surface: 115-170 foot-candles in one room, 500-680 in another, and 800-1300 in the third. A unit of

ten plants was removed from each room 1 hour after the plants had been placed therein, and each hour thereafter, 20 hours being required to remove all the plants. Each unit removed was placed in the darkroom and left there for 12 hours, after which it was returned to a control room maintained at 23° C., where it received 2000-3000 foot-candles at the leaf surface. After remaining in this control room for 8 hours or more, the plants were returned to the long-day bench during day-light, where they remained until dissected.

The results of a part of this experiment are given in figure 4. The abscissa indicates the length of the variable photoperiod in hours, and the ordinate, the average of the stage of flowering for the various units of ten plants. Except for a more rapid increase during the first 2 hours of exposure, the stage of floral development of the plants exposed to the photoperiod of highest light intensity was not greatly different from that of those exposed to the medium intensity. For this reason the data for the high intensity are not shown in the graph.

The control unit, the stage of flowering of which is shown at the extreme lower lefthand corner of the graph, contained one flowering and nine vegetative plants. Of the other units containing more than one vegetative plant, the following list gives the length of photoperiod and the number of vegetative plants in each case. At the low intensity there were: 1 hour, eight vegetative; 2 hours, four vegetative; and 6 hours, two vegetative. At the medium intensity there were: 1 hour, three vegetative (nine plants in all); and 2 hours, four vegetative.

The stages of floral development resulting from exposure to these two light intensities are well separated for all photoperiods of the same length: that is, the level of condition A resulting from exposure to the lower intensity shows no tendency to approach the level at the higher intensity, even after prolonged exposure to light.

Figure 4 indicates, as do figures 1 and 3, that the stage of flowering, or the intensity of condition A, increases rapidly with increase in the length of the photoperiod for the first few hours of exposure to light, after which further increase in the photoperiod increases the stage of flowering only slightly. Further, although different light intensities may produce marked differences in the level of A, within wide ranges of light intensity this difference is relatively small.

EXPERIMENT 5: INITIATION AND DEVELOPMENT OF FLOWERS AFFECTED BY
LENGTH OF SINGLE DARK PERIOD FOLLOWING LONG
PHOTOPERIOD OF HIGH LIGHT INTENSITY

Xanthium may be kept indefinitely vegetative if exposed to photoperiods of 18-20 hours with intervening dark periods of 6-4 hours. If one of these cycles is modified by increasing the length of the dark period beyond 8½-9 hours, the plants are induced to flower even though subsequently they may be returned to conditions of long photoperiod or continuous light.

For dark periods around 23° C. the minimum length of the dark period necessary to induce flowering is $8\frac{1}{2}$ –9 hours. It has been shown (experiment 8) that several photoinductive cycles with long dark periods are more effective in the induction of flower primordia—and especially in the development of flowers—than a single cycle. That the effectiveness of a single long dark period might be augmented by increasing it beyond the $8\frac{1}{2}$ –9 hours' minimum length necessary to induce flowering is a possibility.

This experiment was carried out at Chicago during the summer of 1939. The plants were grown on the long-day bench from June 2 until June 30. Following a bright day, units of ten plants each were taken from the long-day bench and given dark periods of various lengths, as indicated in figure 5. Following this dark period the units were exposed to either of two conditions: part of them were returned to

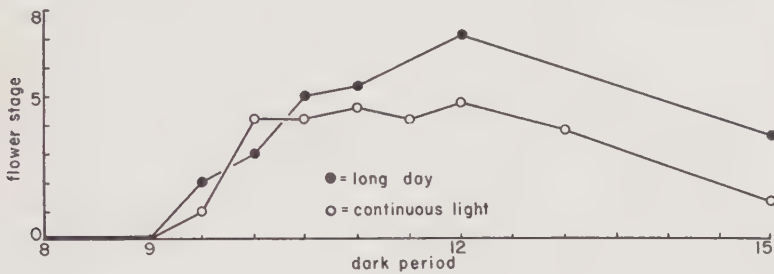


FIG. 5.—Effect of length of single dark period and subsequent photoperiodic treatment on initiation and development of flower primordia in *Xanthium*. Plants received single photoinductive cycle.

the long-day bench and part were placed under continuous light (daylight extended by Mazda filament light of about 100 foot-candles from sundown to sunrise). As indicated by unpublished experiments, and as found by HAMNER (3), long photoperiods and short dark periods following flower induction are more favorable to flower development than is continuous light.

The results of this treatment are shown in figure 5. The abscissa indicates the length of the single long dark period to which the plants were exposed; the ordinate indicates the relative amount of initiation and floral development. One line in the graph represents those plants returned to long-day conditions to develop; the other line, those which remained on continuous light. As noted under the section on methods, the scale for stage of flowering used here differs from that in experiments 1–4 and 8.

Each experimental unit consisted of ten plants. Under the conditions of this experiment all plants given dark periods of $8\frac{1}{2}$ –9 hours remained strictly vegetative. Under usual experimental conditions, at least some plants receiving a dark period of this length would flower. This and the relatively large numbers of vege-

tative plants listed below probably resulted from the removal of the plants from the dark period into early morning light of rather low intensity (see experiment 6). Among those units returned to long-day conditions after treatment, the following contained more than one vegetative plant: 9.5 hours, six vegetative; 10 hours, four vegetative; 15 hours, four vegetative. Among those lots returned to continuous light after treatment, the following contained more than one vegetative plant: 9.5 hours, seven vegetative; 10 hours, two vegetative; 11 hours, three vegetative; 11.5 hours, three vegetative; 13 hours, two vegetative; 15 hours, three vegetative.

This experiment has been repeated twice, employing dark periods up to 50 hours and allowing the plants to develop on long photoperiods. Both repetitions indicate a maximum effective length of 12-15 hours for the dark period.

EXPERIMENT 6: INITIATION AND DEVELOPMENT OF FLOWERS AFFECTED BY
PHOTOPERIODS OF LOW LIGHT INTENSITY IMMEDIATELY
PRECEDING AND FOLLOWING LONG DARK PERIOD

For *Xanthium* plants kept continuously vegetative by growing under conditions of long day, the length of the dark period is the main factor limiting the induction

TABLE 1

EFFECT OF LOW LIGHT INTENSITY ON INITIATION AND DEVELOPMENT OF FLOWERS

TREATMENT		AVERAGE STAGE OF FLOWERING	NO. OF PLANTS	
PHOTOPERIOD PRECEDING 12-HOUR DARK PERIOD	PHOTOPERIOD FOLLOWING 12-HOUR DARK PERIOD		VEG.	FL.
12 hours of 200 f.c. Mazda filament light	{ 12 hours of 200 f.c. Mazda filament light.	0.0	20	0
	{ 12 hours of 1200 f.c. arc light.	0.3	18	2
12 hours of 1200 f.c. arc light	{ 12 hours of 200 f.c. Mazda filament light.	0.15	16	4
	{ 12 hours of 1200 f.c. arc light.	3.5	0	20

of the flowering condition. Under usual growing conditions the lengthening of a single dark period beyond $8\frac{1}{2}$ –9 hours will bring about initiation. With more careful consideration of the rate of development of primordia, other factors affecting the relative strength of a photoinductive cycle are found in addition to the length of the photoperiod or dark period. A number of these have been indicated in the previous experiments. The intensity of the light of the photoperiod immediately preceding and/or following a long dark period is of considerable importance.

Selected plants were given a single dark period of 12 hours preceded and followed by 12-hour photoperiods of controlled light intensity. On October 7 eighty plants from the long-day bench were divided into two lots of forty each, one lot receiving Mazda filament light at an intensity of 200 foot-candles and the other re-

ceiving arc light of about 1200 foot-candles, for 12 hours; then each lot received a 12-hour dark period. At the end of this dark period, half the plants in each of the two treatments were returned to the light of 200 foot-candles for 12 hours, and the remaining half were placed under the light of 1200 foot-candles. At the end of this treatment the four groups were returned to the long-day bench, where they remained until harvested for dissection, 27 days after the start of the experiment. The method of measuring the flower stage in the following data is different from that used in experiments 1-4 and 8. The nature of the treatments and the data are summarized in table 1.

EXPERIMENT 7: INITIATION AND DEVELOPMENT OF FLOWERS BY PHOTO-INDUCTIVE CYCLES WITH LOW LIGHT INTENSITY PHOTOPERIOD

Experiment 6 indicated that decreasing the intensity of light preceding and following a long dark period markedly decreased the effectiveness of that dark

TABLE 2
EFFECT OF PHOTOPERIODS OF LOW LIGHT INTENSITY
ON INITIATION AND DEVELOPMENT OF FLOWERS

NO. OF PHOTO- PERIODS, EACH SEPARATED BY 12-HOUR DARK PERIOD	LIGHT INTENSITY IN F.C. DURING PHOTOPERIOD	AVERAGE STAGE OF FLOWERING	NO. OF PLANTS	
			VEG.	FL.
2	18.....	0.0	9	0
	50.....	0.0	10	0
	100.....	0.0	10	0
11	18.....	1.4	3	4
	50.....	1.6	2	8
	100.....	2.3	1	6

period in inducing flower primordia. However, a photoperiod of very low intensity may be effective in bringing about induction of flowers if the photoinductive cycles are repeated several times.

At 8:00 A.M., October 6, sixty plants were removed from the long-day bench, and two units of ten plants each were placed under each of three intensities of Mazda filament light—18, 50, and 100 foot-candles. After a 12-hour exposure, all plants received a 12-hour dark period. This dark period was followed by an exposure to 12 hours of light of the same intensity to which the unit was first exposed. Half the lots were then returned to the long-day bench, where they remained until dissected, while the remaining half received nine more of these cycles before being returned to the bench. All plants were dissected November 4. The experimental procedure and results are presented in table 2.

EXPERIMENT 8: EFFECT OF NUMBER OF PHOTOINDUCTIVE CYCLES ON RATE
OF DEVELOPMENT OF FLOWERS AND FRUITS GROWN UNDER
CONDITIONS OF LONG DAY

Table 3 shows in a general way the effect which the number of induction periods has on the rate of development of flower structures and fruits. The first visible evidence of inflorescence initiation appears 2-3 days following a single induction period of the type given here. For this reason the effect of the number of inductive

TABLE 3

NUMBER OF CYCLES EACH CONSISTING OF A 12-HOUR PHOTOPERIOD FOLLOWED BY A 12-HOUR DARK PERIOD. EXPERIMENT STARTED THE MORNING OF APRIL 2. ALL FIGURES WITHOUT QUALIFYING TERMS REFER TO STAGE OF FLOWERING

DATE EXAMINED	CYCLES				CONTINUOUSLY REPEATED
	1	2	4	8	
April 5.....	1.0	1.0	1.0		
April 8.....	2.6	3.6	4.6	4.8	
April 11.....	3.3	9.1	12.3	11.0	
April 14.....	4.4	12.3	17.4	18.7	
April 17.....	7.4	13.4	23.3	33.3	
April 22.....	9.4	13.8	24.0	43.8	
April 24.....		18.0	30.5	Pollen first shed	Pollen being shed
April 29.....	11.4	18.8	44.8	Bur length 14.2 mm.	Bur length 20.5 mm.
May 6.....			Pollen first shed		
May 8.....	17.8	28.3			
June 4.....	Pollen being shed	Bur length 12.2 mm.	Bur length 33.3 mm.	Burs nearly mature, becoming brown	Burs completely mature, length 22.4 mm.; plants dead

cycles is much less evident in earlier stages of development of inflorescence and flower primordia than in the later stages. The later development proceeds much more rapidly in plants which received several short day treatments than in plants which received only one or two such treatments.

Plants were selected April 2 from a group seeded on the long-day bench February 20. The cycles used for flower induction consisted of a 12-hour photoperiod of natural daylight in the greenhouse (6:00 A.M. to 6:00 P.M.) followed by a 12-hour dark period. The various lots received 1, 2, 4, 8, and continuously repeated cycles. All lots except the last were returned to the long day bench for development after treatment. The stage of flowering is measured on the same scale as that used for experiments 1-4.

By June 4, 64 days after induction began, the plants on continuous short day were completely mature and had died. At the other extreme were those plants which had received only one long night and were just beginning to shed pollen. Plants developing on continuous short days bore fruit which differed markedly from that of plants induced with a few short days and then allowed to develop on long days. The burs of the former are much smaller, bear only a few spines (in comparison with those on long day), and frequently the thin involucre is broken, allowing the ovary to protrude for half its length.

Discussion

The effect of temperature during the dark period may be compared with that during the photoperiod. LONG (4) has shown that lowering the temperature increases the critical length of the dark period. The temperature during the dark period is also of importance in flower induction in Biloxi soybeans (5). It has been previously found with *Xanthium* that temperature had little effect on the photoperiodic reaction (4). Experiment 3, however, shows definite temperature effect. For short photoperiods this effect was pronounced, but it decreased as the photoperiods increased in length. Previous workers, when considering the relative effects of temperature on the dark period and the photoperiod, have used dark periods near the critical length but photoperiods far in excess of the minimum length. This probably explains the resultant relatively low coefficient of temperature for the reactions of the photoperiod. In Biloxi soybean the effect of temperature was found to be slight but significant (5).

In comparing the results of these experiments with other data, especially from other species, it must be kept in mind that the flowering responses in experiments 1-6 depended upon variable factors introduced into a single photoinductive cycle. The plants were exposed to only one cycle during which flower induction could have taken place. Many short-day plants, such as Biloxi soybean, require several cycles of treatment for such induction. LONG (4) has shown that when several cycles are given, the time elapsing between consecutive cycles is of great importance. This spacing may determine in part the most effective length of photoperiod or dark period for induction of flowers, as has been suggested by HAMNER (2). Thus in experiment 5 the optimum length for the dark period was found to be 12-15 hours, although this would probably be decreased if several cycles were given instead of one, since long dark periods space the photoinductive cycles farther apart. When several photoinductive cycles are given, this effect must be considered.

The importance of the light period immediately following a dark period has been neglected in most work with *Xanthium*. Experiment 6 indicates that the photoperiod following a long dark period—as well as that preceding it—may be impor-

tant. When plants are exposed to several consecutive photoinductive cycles, a photoperiod both follows and precedes a dark period. The role of the photoperiod under these conditions may be somewhat different from a single-cycle experiment, where the test photoperiod only precedes a dark period. Experimental work on the effect of light immediately following a dark period is needed.

In the preceding experiments reference has been made to condition A, which has been defined as "the changes or conditions which arise owing to exposure to light" (2). Such changes or conditions are those which develop on exposure to high light intensity at moderate temperatures. Within certain limits the intensity of A increases with increasing exposure to light. In addition to its function of establishing condition A, but probably distinct from it, light interferes with the reactions which occur during the dark period following a photoperiod. Here continuous light of very low intensity or periods of moderate intensity and of very short duration are effective. In *Xanthium*, 1 minute of light in the middle of a 9-hour dark period will nullify the effect of the dark period so far as the initiation of flowers is concerned. In Biloxi soybean, BORTHWICK and PARKER (1) have demonstrated that Mazda light of 0.6 foot-candles, given for the first 8 hours of a 16-hour dark period, will prevent flower induction.

In *Xanthium* and Biloxi soybean condition A is developed most rapidly under light of high intensity. On the other hand, development of condition B depends on the exclusion of light, 0.6 foot-candles being sufficient to inhibit this condition in the soybean. There is nothing to indicate that the reactions brought about by light of high intensity (the establishment of condition A) are the same as those which occur when light interferes with the reactions taking place in the dark.

If two distinct reactions or groups of reactions are present, the recognition of this fact is of some importance. WITHROW and BIEBEL (6), using salvia, a short-day plant, found that flowering plants would return to the vegetative state if placed on long photoperiods. Giving these plants a short photoperiod of natural daylight and extending this with additional low light intensity also caused the plants to revert to the vegetative condition; that is, the short photoperiod supplemented by low light intensity brought about a response characteristic of long photoperiods. At low intensities red radiation was found to be more effective as supplementary light than other portions of the spectrum used. They conclude that, "under the conditions of the experiment, red radiation is most effective in producing the photoperiodic response both in long and short day plants." It would seem that, with respect to short-day plants, their experiments dealt with the second mentioned response to light rather than with the establishment of condition A, and indicate that red radiation is most effective in interrupting the dark period—in interfering with condition B.

Summary

1. Induction of flowers may take place in vegetative plants of *Xanthium pennsylvanicum* when subjected to a single controlled photoinductive cycle. This flowering response is recorded on a more extensive scale than has previously been used. The measurement is based on the size of the apical inflorescence.

2. The effect of the length of the photoperiod given during this single cycle is related both to its temperature and to its light intensity. In general, the initiation of flower primordia and their rate of development increase with the increasing length of the photoperiod up to a rather definite point, beyond which further lengthening of the photoperiod has little effect.

3. By increasing the light intensity, the effectiveness of the photoperiod of the single photoinductive cycle is increased, at least for large differences of light intensity. This response seems independent of the length of the photoperiod, being evident even after 20 hours. Photoperiods of as low as 18 foot-candles may bring about induction if repeated consecutively several times.

4. The temperature during the photoperiod has a pronounced effect on the resultant stage of flowering. At 10° C. a single photoperiod of 2000–3000 foot-candles must be in excess of 4–5 hours to cause flowering. At 30° C. a photoperiod of similar intensity but only $\frac{1}{2}$ hour in length may cause initiation. As the photoperiod increases in length, the effect of the temperature diminishes.

5. In plants induced to flower by increasing the length of a single dark period beyond 8½–9 hours, the maximum stage of flowering is brought about by dark periods of 12–15 hours.

6. The photoperiod following a long dark period—as well as that preceding it—has a pronounced effect on the initiation and development of flowers.

7. Data are given showing the influence of the number of photoinductive cycles to which plants are exposed on the rate of development of flower structures.

8. Some of the data of these experiments are interpreted on the basis of HAMNER's suggestion that the induction of certain short-day plants may be represented by A, B→C.

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LITERATURE CITED

1. BORTHWICK, H. A., and PARKER, M. W., Photoperiodic perception in Biloxi soybeans. BOT. GAZ. 100:374–387. 1938.
2. HAMNER, K. C., Interrelation of light and darkness in photoperiodic induction. BOT. GAZ. 101:658–687. 1940.

3. HAMNER, K. C., and BONNER, JAMES, Photoperiodism in relation to hormones as factors in floral initiation and development. *BOT. GAZ.* **100**:388-431. 1938.
4. LONG, E. M., Photoperiodic induction as influenced by environmental factors. *BOT. GAZ.* **101**:168-188. 1939.
5. PARKER, M. W., and BORTHWICK, H. A., Effect of variation in temperature during photoperiodic induction upon initiation of flower primordia in Biloxi soybean. *BOT. GAZ.* **101**:145-167. 1939.
6. WITHROW, R. B., and BIEBEL, J. P., Photoperiodic response of certain long and short day plants to filtered radiation applied as a supplement to daylight. *Plant Physiol.* **11**:803-819. 1936.

